exposure influence disease pathogenesis. Studies using high-fat, low-fiber Western diet have demonstrated major alterations in the microbial composition in the gut, characterized by decrease in the abundance of butyrate-producing species. Therefore, the current study potentially highlights the mechanistic link between increase in T1D incidence and changes in diet in Westernized societies.

There is a rising interest in the functional impact of microbial metabolites on host physiology. Major challenges in the field include determining relevant sites of sampling, better sensitivity for detection, and accurate identification of the metabolites. To date, fecal metabolites have been most commonly examined, but there is an increasing awareness for the need to examine metabolites from tissue-associated microbes. Although mass spectrometry and NMR techniques have been useful in detecting and identifying metabolites, a substantial number of candidate metabolites have not been annotated. Also, current genetic modification techniques and robust culture methods exist only for a fraction of the intestinal bacteria that have been mapped, and the standard experimental approaches to study the function of microbiota involve removal and recolonization of those culturable bacteria. Even with a refined experimental system, it is challenging to distinguish the source of metabolites as host cells and microbes utilize many common metabolic pathways. Sun et al. (2015) propose that butyrate directly signals on β-cells as they express G protein-coupled receptors (GPR) 41 and 43, the two receptors for SCFA. Although addition of butyrate to islet culture potently induced CRAMP expression in vitro, butyrate was not detectable in the islets of healthy mice at baseline in vivo. Although the luminal concentrations of SCFA are ~50 mM, systemic concentrations are much lower (~1–10 μm). Furthermore, systemic injection of supra-physiologic doses of butyrate might not accurately represent in vivo conditions. Their data suggest that the immunoregulatory effect of butyrate in T1D is CRAMP-dependent, though one cannot rule out the role of CRAMP-independent differentiation of Tregs and regulatory macrophages directly mediated by butyrate. Therefore, future experiments are required to prove that such a direct effect occur in vivo and whether homeostatic levels of systemic SCFA confer protection against the disease onset in healthy mice. Despite these caveats, this study is an important step forward.

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Memory NK Cells Take Out the (Mitochondrial) Garbage

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The molecular mechanisms important to generate innate natural killer cell "memory" are poorly understood. In this issue of *Immunity*, O'Sullivan et al. (2015) demonstrate that mitophagy plays a critical role in natural killer cell memory formation following viral infection.

The mammalian immune system is classically divided into rapid, non-specific innate immunity and slower-developing, antigen-specific adaptive immunity that exhibits immunologic memory. Memory in immune lingo conventionally denotes a more robust, rapid secondary response to a previously encountered "priming" infectious challenge. Natural killer (NK) cells are innate lymphoid cells important for host defense against viral infection and malignancy, which is primarily achieved via direct target cell killing and the production of effector cytokines (e.g., IFN-γ) and chemokines. Unlike adaptive T and B lymphocytes that recombine the DNA of antigen receptor genes to express a dominant, antigen-specific activating

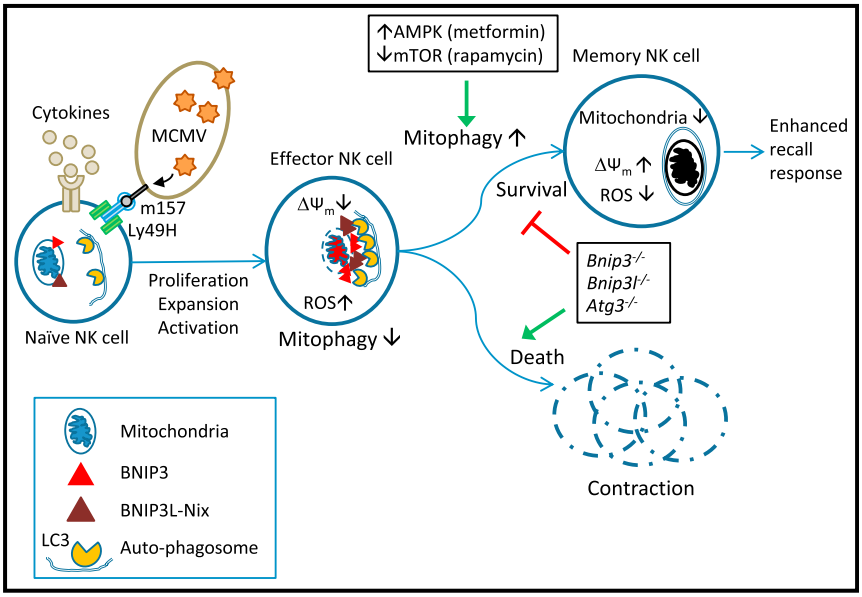

Figure 1. Mitophagy Is Required to Generate a Functional Memory NK Cell Compartment after MCMV Infection

Acute infection with MCMV results in the proliferation, expansion, and activation of effector NK cells during the first 5–7 days post-infection. A decrease in mitophagy during this effector NK cell formation leads to an accumulation of dysfunctional mitochondria and increased amounts of mitochondrial-associated reactive oxygen species. Key events that induce mitophagy are required to generate and preserve memory NK cells during the contraction of effector NK cells, including induction of mitochondrial receptors BNIP3 and BNIP3L that recruit autophagosomes to eliminate damaged mitochondria, facilitating cell survival. Experimental conditions that result in enhanced memory NK cell survival include promotion of mitophagy or autophagy by inhibition of mTOR or activation of AMPK. The requirement for mitophagy was demonstrated by showing that NK cells deficient in the pro-mitophagy proteins Bnip3 or Bnip3l, or the autophagosome component Atg3, have markedly decreased memory NK cells. Abbreviations are as follows: ROS, reactive oxygen species; ΔΨm, mitochondrial membrane potential; mTOR, mammalian target of rapamycin; AMPK, AMP activated protein kinase.

receptor, NK cells express numerous germline-encoded activating and inhibitory receptors whose signals integrate to govern NK cell responses to target cells. Thus, NK cells are capable of responding to a broad range of activating receptor ligands and/or loss of major histocompatibility complex (MHC) class I in the setting of disease. NK cell diversity results largely from the variable expression of these receptors on individual NK cells, resulting in thousands of NK cell "subsets" with distinct receptor repertoires. NK cells have traditionally been classified as "innate" since they lack a recombined antigen-specific receptor, respond rapidly to target cells, and were not thought to retain a memory of prior activation. However, this paradigm has been challenged by several studies in mice identifying adaptive aspects of the NK cell response that include both specificity and innate memory (Min-Oo et al., 2013).

NK cell immune memory-like responses have been reported following exposure to haptens (O'Leary et al., 2006), cytokine activation (Cooper et al., 2009), and viral infections such as murine cytomegalovirus (MCMV) (Sun et al., 2009)—the focus of this Preview. Host defense against MCMV requires specific recognition of the MCMV-encoded protein m157 by the activating Ly49H receptor on NK cells. In this model infection, Ly49H$^+$ NK cells proliferate, expand, and become highly activated during acute MCMV infection, a response that is initially driven by cytokines and later specifically via Ly49H-m157 interaction. Following resolution of viremia, the effector NK cell population contracts, and the remaining NK cells are enriched in Ly49H$^+$ NK cells for months. Utilizing adoptive transfer of Ly49H$^+$ NK cells into Ly49H-deficient hosts, Sun et al. demonstrated that virus-specific NK cells from MCMV-experienced mice exhibit secondary expansion, enhanced effector responses, and superior protection against MCMV infection, compared to Ly49H$^+$ NK cells from naive, uninfected mice (Sun et al., 2009). In light of similarities to the memory CD8$^+$ T cell anti-viral response, these long-lived NK cells with enhanced recall responses upon re-stimulation were termed "memory" NK cells. Several critical, non-redundant mechanisms have been identified in MCMV-induced NK cell memory formation including interleukin-12 receptor signaling, the transcription factor Zbtb32 that promotes NK cell proliferation by inhibiting the tumor suppressor Blimp-1, signaling by the DNAM-1 costimulatory receptor, and influence of the pro-apoptotic protein Bim (O'Sullivan and Sun, 2015). Despite these important advances in this nascent aspect of NK cell biology, our overall understanding of the molecular mechanisms required for innate memory formation remains limited. This present study by O'Sullivan et al. (2015) sheds light on new mechanisms by demonstrating that mitophagy during the contraction of an MCMV-induced effector NK cell population is crucial for the differentiation of a stable memory NK cell pool.

Mitophagy is the selective autophagic degradation of mitochondria, whereby double-membraned vesicles (autophagosomes) engulf a target organelle and fuse with lysosomes, degrading and recycling the sequestered contents (Green et al., 2011). Mitophagy allows for the elimination of damaged or dysfunctional mitochondria, which otherwise cause oxidative stress and cellular injury, thereby inducing apoptotic cell death. Autophagy, including the elimination of dysfunctional mitochondria, has been shown to play an essential role in CD8$^+$ memory T cell development (Xu et al., 2014). In the present study, mitophagy was also shown to be important in MCMV-induced memory NK cell formation using in vivo gain- and loss-of-function approaches. Moreover, key molecular players were identified that were not previously known to orchestrate this process in memory-type lymphocytes (O'Sullivan et al., 2015).

O'Sullivan et al. first show using several methods that MCMV-driven expansion of effector Ly49H$^+$ NK cells resulted in the accumulation of dysfunctional mitochondria and autophagosomes. However, after 7 days when effector NK cells contract leading to memory NK cell generation, mitochondrial quality was improved and overall mitochondrial numbers decreased, correlating with a decline in

autophagosomes. These findings support a model where dysfunctional mitochondria that accumulate in effector NK cells are cleared by mitophagy, allowing for differentiation of MCMV-specific memory NK cells (Figure 1). The authors demonstrated a requirement for autophagic activity during the formation of MCMV-specific memory NK cells using mice with a tamoxifen-inducible deletion of *Atg3*, which encodes an essential autophagosome component. Deletion of *Atg3* during the proliferative phase following MCMV infection did not impact the expansion of Ly49H$^+$ NK cells; however it resulted in a marked reduction of the ensuing pool of memory NK cells. While compelling, the experimental evidence provided leaves open the possibility that *Atg3* deletion per se might result in reduced NK cell homeostasis, regardless of this specific memory context. Providing further support for their hypothesis, O'Sullivan et al., showed that by enhancing autophagy, through the inhibition of mTOR or activation of AMP activated protein kinase, an expanded number of memory NK cells was generated. Autophagy was connected to the clearance of dysfunctional mitochondria, since increased mitochondrial numbers, poor mitochondrial quality, and higher amounts of mitochondrial-associated reactive oxidative species (ROS) were seen during the transition from effector to memory type cells in NK cells lacking *Atg3* (O'Sullivan et al., 2015). Mitochondrial dysfunction was linked to the survival defect of Atg3-deficient memory NK cells since this phenotype was rescued by N-acetylcysteine, a ROS scavenger. BNIP3 and BNIP3L (Nix) are mitochondrial receptors that promote mitophagy by recruiting the autophagosome via binding to LC3 proteins. O'Sullivan et al. also demonstrated that deletion of either BNIP3 or BNIP3L resulted in accumulation of dysfunctional mitochondria, impaired mitophagy, and a markedly decreased MCMV-specific memory NK cell pool. Importantly, deletion of BNIP3 or BNIP3L did not affect normal NK cell development or function following homeostatic proliferation, highlighting the importance of mitophagy specifically during memory NK cell formation. These findings also lead to new mechanistic questions of how BNIP3 and BNIP3L are regulated in NK cells to selectively induce mitophagy during a memory response. Is there a direct connection between mitophagy and antigen-specific NK cell responses? Further, do other types of T and NK cell memory, or other aspects of NK cell biology, depend upon (or are they limited by) autophagy or mitophagy?

In summary, this study identifies a novel requirement for mitophagy in the formation of virus-induced NK cell memory and pinpoints BNIP3 and BNIP3L as key mechanistic players not previously implicated in lymphocyte memory formation. Thus, the hypothesis put forward by the authors that the need to "take out the garbage" of damaged mitochondria might be a general hallmark of immunologic memory is intriguing and warrants further study in all adaptive lymphocytes.

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ILC Lineage Specification: To Be or Not 11b, That Is the Question

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The transcription factor Bcl11b is important for T cell development and maintaining their phenotype. In this issue of *Immunity*, Califano et al. (2015) show that Bcl11b has a role in specifying type II innate lymphoid cell (ILC2) identity and blocks their conversion to ILC3s.

It is a remarkable testament to the speed of progress in modern biological research that it has been only 6 or 7 years since the first inklings emerged of the existence of a hitherto unknown subset of lymphocytes (Satoh-Takayama et al., 2008; Cella et al., 2009; Luci et al., 2009). Now these cells are defined as innate lymphoid cells (ILCs) with an ever-expanding set of critical functions within the immune system (Eberl et al., 2015). ILCs have come to be seen as ersatz T cells for innate